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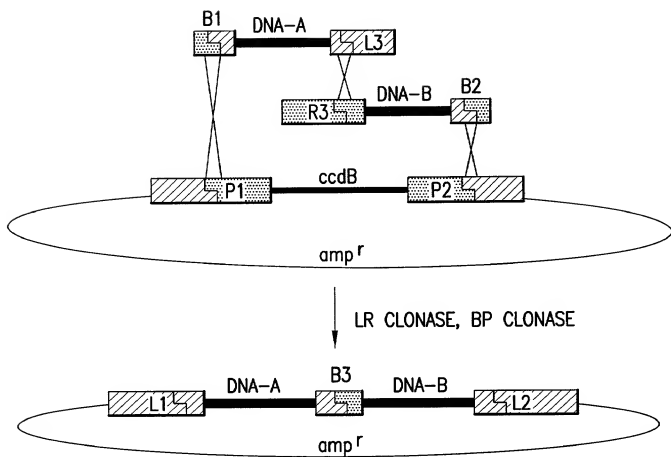


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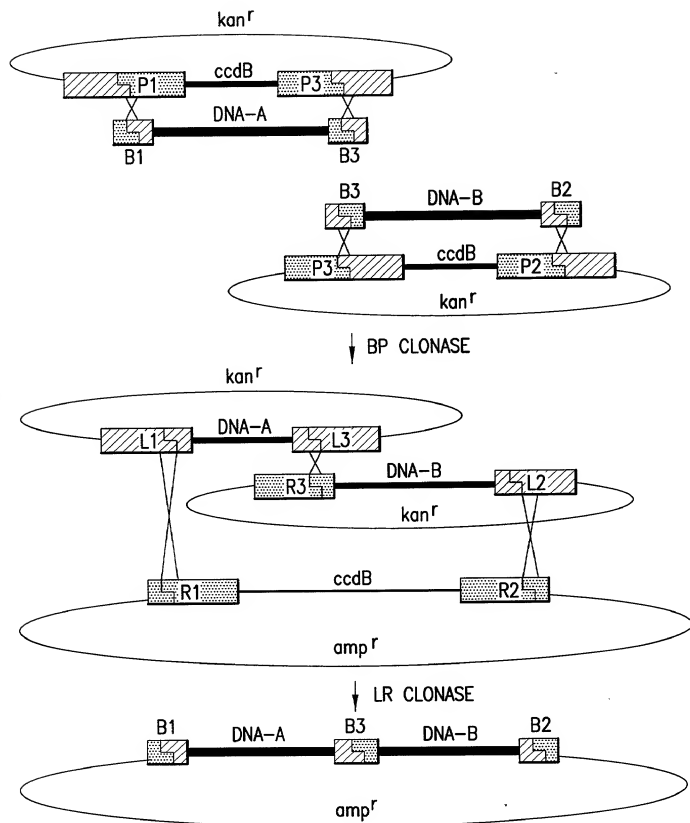


FIG.4

1. The first step is to create a DNA-A fragment with a B1 site and a P3 site. The B1 site is a recognition site for the B1 restriction enzyme, and the P3 site is a recognition site for the P3 restriction enzyme. The DNA-A fragment is also flanked by a ccdB gene and a kan<sup>r</sup> gene.

2. The second step is to create a DNA-B fragment with a B3 site and a B2 site. The B3 site is a recognition site for the B3 restriction enzyme, and the B2 site is a recognition site for the B2 restriction enzyme. The DNA-B fragment is also flanked by a ccdB gene and a kan<sup>r</sup> gene.

3. The third step is to create a DNA-C fragment with a P1 site and a P2 site. The P1 site is a recognition site for the P1 restriction enzyme, and the P2 site is a recognition site for the P2 restriction enzyme. The DNA-C fragment is also flanked by a ccdB gene and a kan<sup>r</sup> gene.

4. The fourth step is to perform a BP CLONASE reaction. This reaction results in the formation of a DNA-A fragment with a L1 site and a L3 site. The L1 site is a recognition site for the L1 restriction enzyme, and the L3 site is a recognition site for the L3 restriction enzyme. The DNA-A fragment is also flanked by a ccdB gene and a kan<sup>r</sup> gene.

5. The fifth step is to perform a LR CLONASE reaction. This reaction results in the formation of a DNA-A fragment with a L1 site and a L2 site. The L1 site is a recognition site for the L1 restriction enzyme, and the L2 site is a recognition site for the L2 restriction enzyme. The DNA-A fragment is also flanked by a ccdB gene and a kan<sup>r</sup> gene.

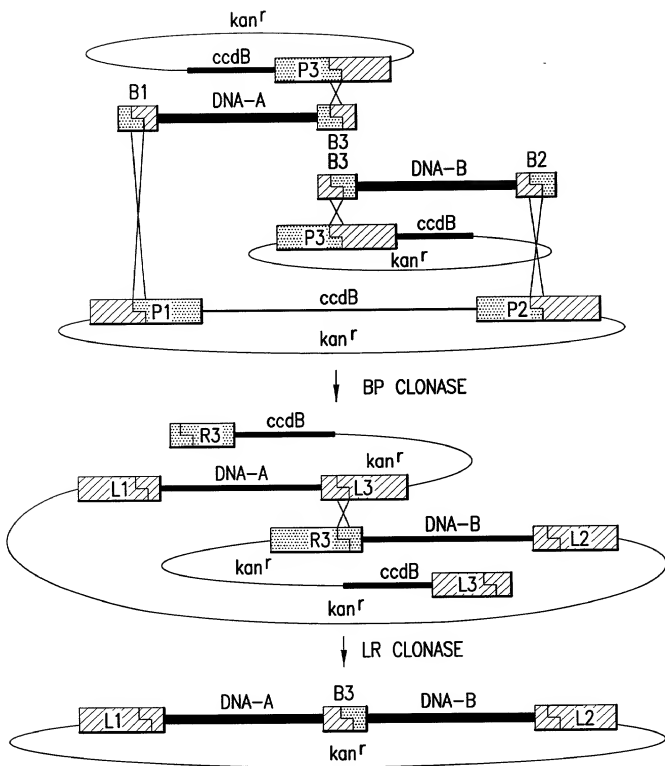


FIG.5

FIG. 6

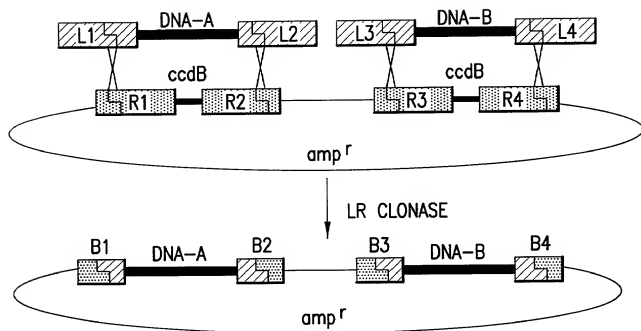


FIG.6

FIG. 7

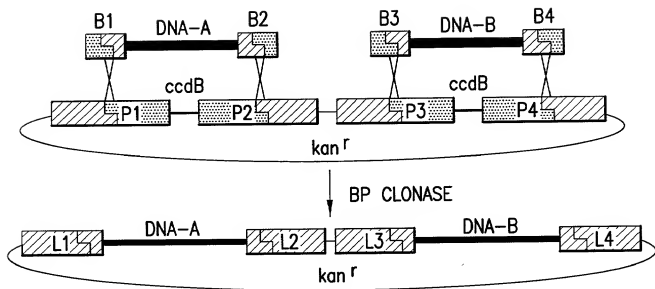


FIG.7

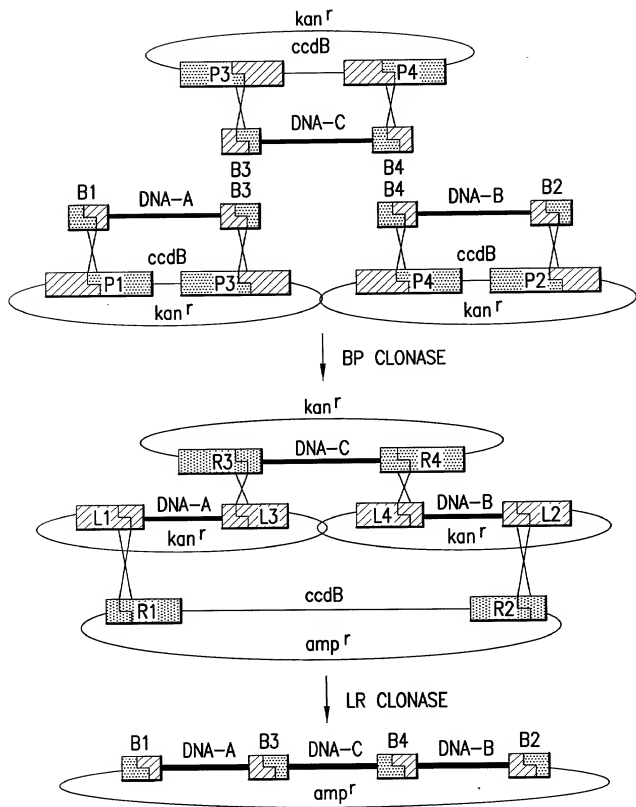


FIG.8





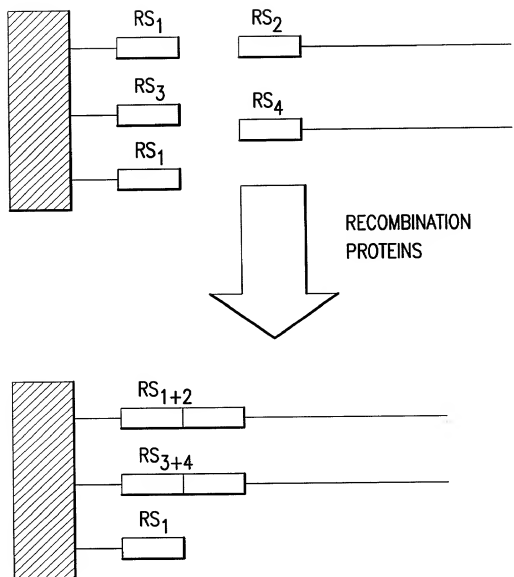
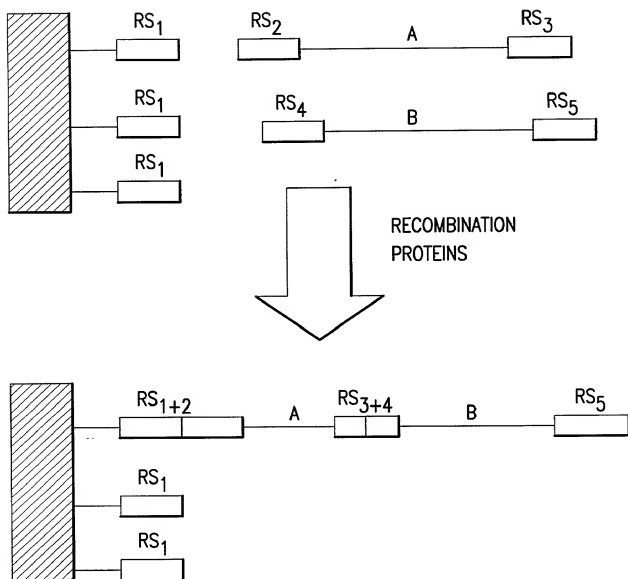


FIG.10



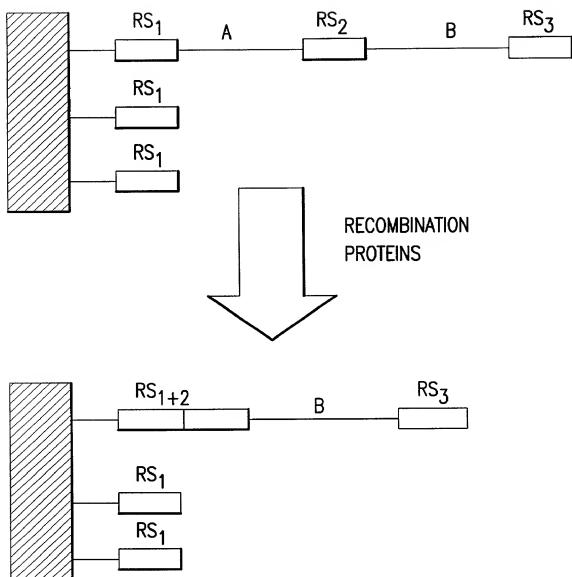


FIG.12

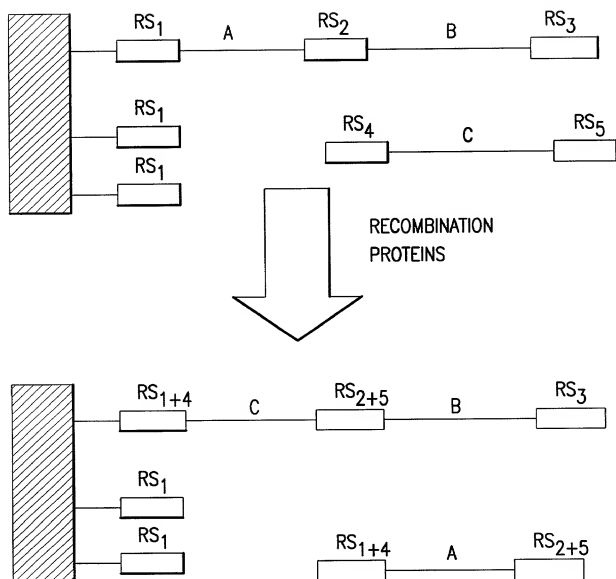


FIG.13

FIG. 14A

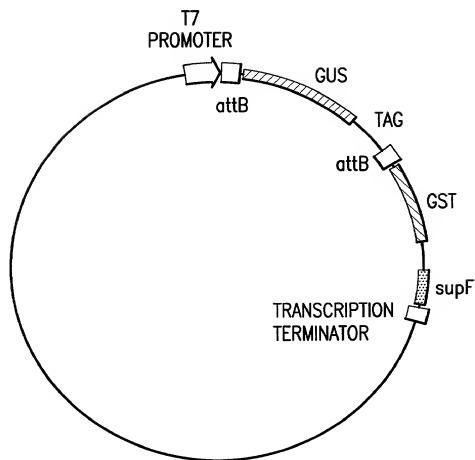


FIG.14A

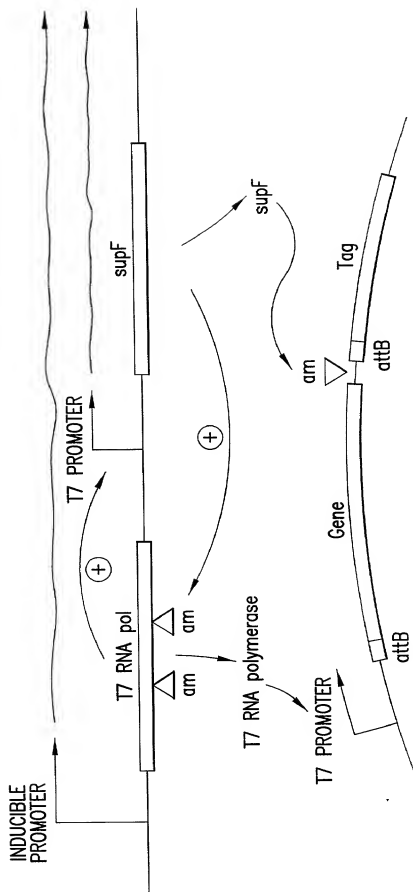


FIG.14B

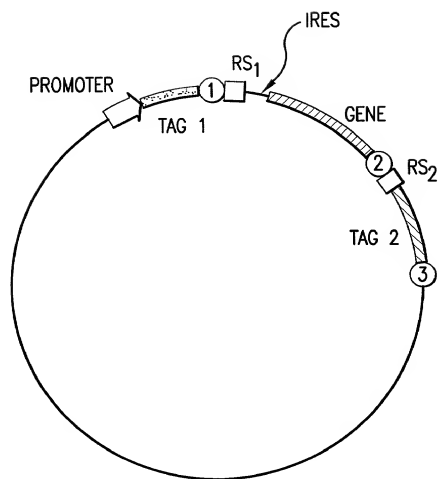
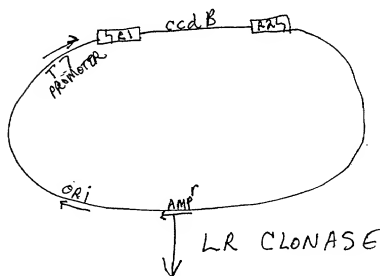
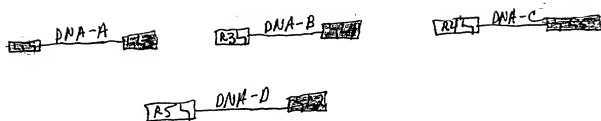


FIG.15



FIGURE 16



LR CLONASE

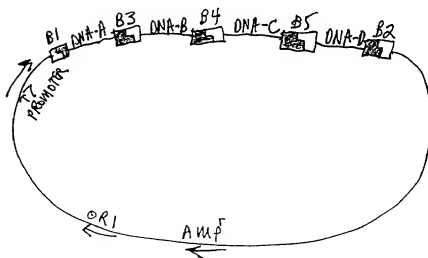


FIGURE 17A

# Cloning Light

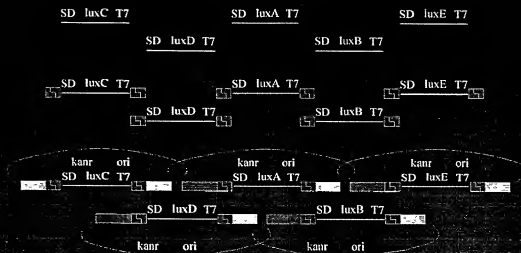


FIGURE 17B

# Cloning Light

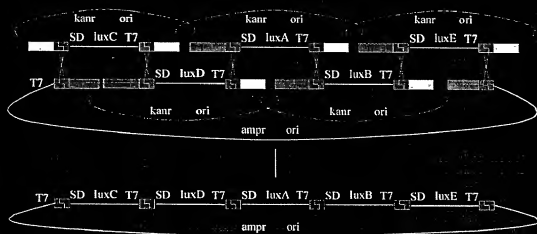


FIGURE 18

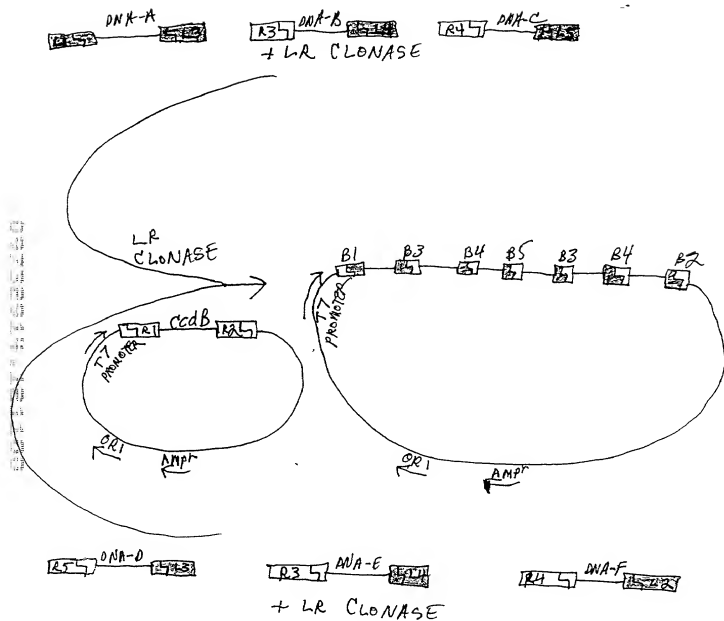


FIGURE 19

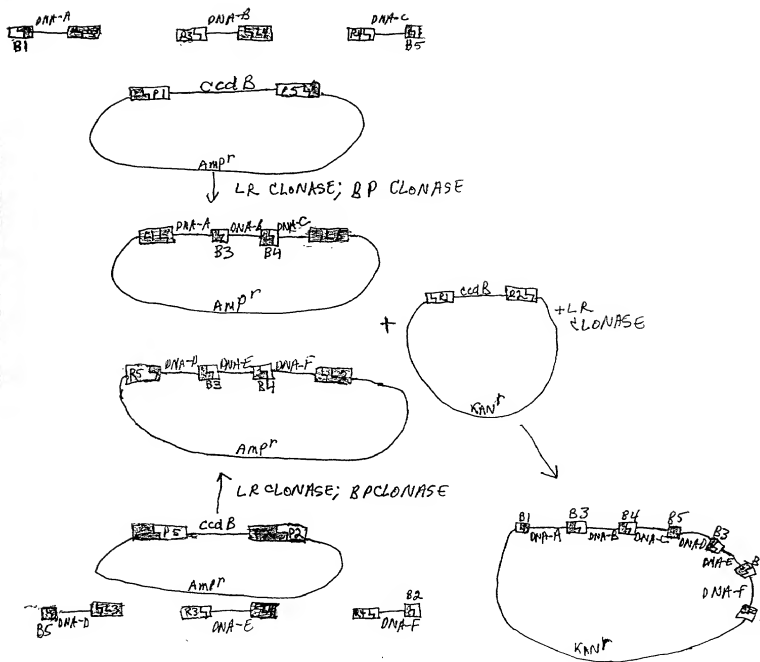


FIGURE 20

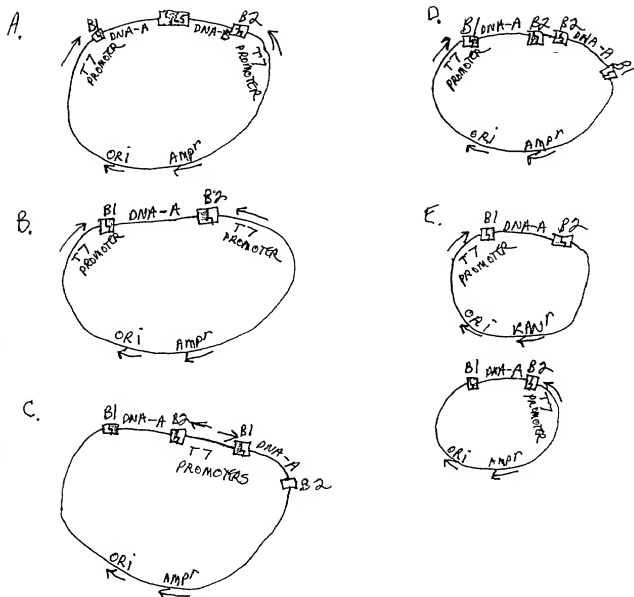


FIGURE 21

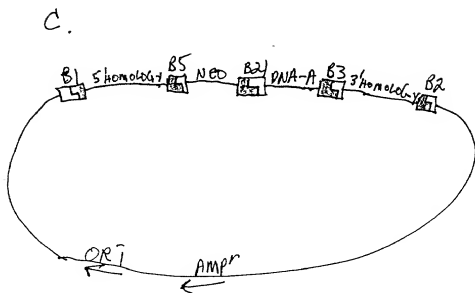
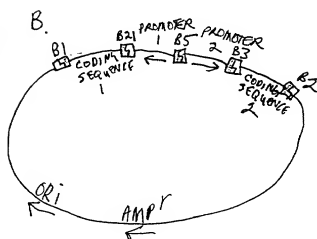
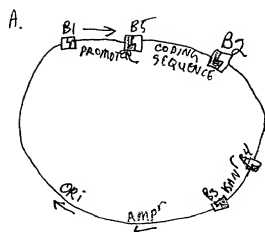


FIGURE 22A

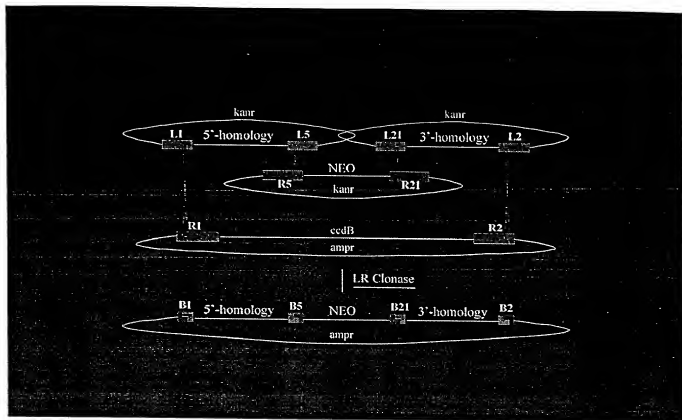
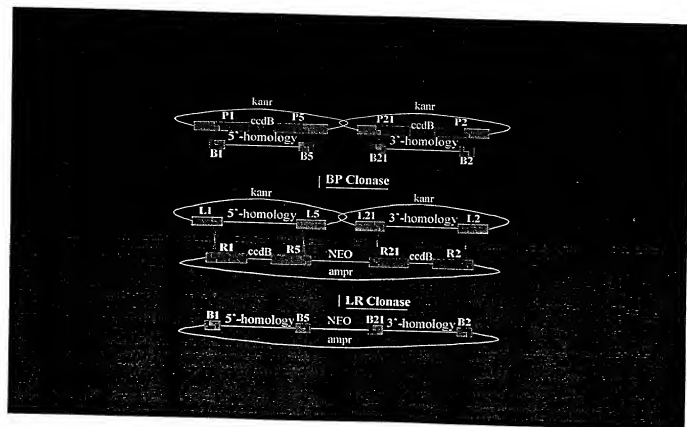
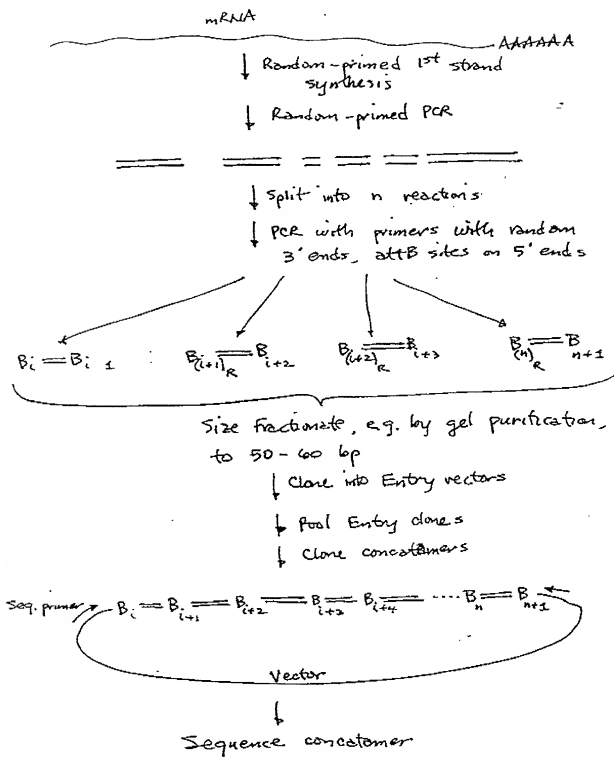


FIGURE 22B



# FIGURE 23





# FIGURE 24A

attB0 AGCCTGCTTTTTTTATACTAACTTGAGC (SEQ ID NO:1)  
TCGGACGAAAAAATATGATTGAACTCG

attP0 GTTCAGCTTTTTTTATACTAAGTTGGCA (SEQ ID NO:2)  
CAAGTCGAAAAAATATGATTCAACCGT

attL0 AGCCTGCTTTTTTTATACTAAGTTGGCA (SEQ ID NO:3)  
TCGGACGAAAAAATATGATTCAACCGT

attR0 GTTCAGCTTTTTTTATACTAACTTGAGC (SEQ ID NO:4)  
CAAGTCGAAAAAATATGATTGAACTCG

---

attB1 AGCCTGCTTTTTTTGTACAACTTGT (SEQ ID NO:5)  
TCGGACGAAAAAATATGTTTGAACA

attP1 GTTCAGCTTTTTTTGTACAAAGTTGGCA (SEQ ID NO:6)  
CAAGTCGAAAAAACATGTTTCAACCGT

attL1 AGCCTGCTTTTTTTGTACAAAGTTGGCA (SEQ ID NO:7)  
TCGGACGAAAAAACATGTTTCAACCGT

attR1 GTTCAGCTTTTTTTGTACAACTTGT (SEQ ID NO:8)  
CAAGTCGAAAAAACATGTTTGAACA

---

attB2 ACCCAGCTTTCTTGTACAAAGTGGT (SEQ ID NO:9)  
TGGGTCGAAAGAATATGTTTCACCA

attP2 GTTCAGCTTTCTTGTACAAAGTTGGCA (SEQ ID NO:10)  
CAAGTCGAAAGAACATGTTTCAACCGT

attL2 ACCCAGCTTTCTTGTACAAAGTTGGCA (SEQ ID NO:11)  
TGGGTCGAAAGAACATGTTTCAACCGT

attR2 GTTCAGCTTTCTTGTACAAAGTGGT (SEQ ID NO:12)  
CAAGTCGAAAGAACATGTTTGACCA

---

attB5 CAACTTTATTATACAAAGTTGT (SEQ ID NO:13)  
GTTGAAATAATATGTTTCAACA

attP5 GTTCAACTTTATTATACAAAGTTGGCA (SEQ ID NO:14)  
CAAGTTGAAATAATATGTTTCAACCGT

# FIGURE 24B

attL5 CAACTTTTATTATACAAAGTTGGCA (SEQ ID NO:15)  
GTTGAAATAATATGTTTCAACCGT

attR5 GTTCAACTTTTATTATACAAAGTTGT (SEQ ID NO:16)  
CAAGTTGAAATAATATGTTTCAACA

---

attB11 CAACTTTTTCTATACAAAGTTGT (SEQ ID NO:17)  
GTTGAAAAGATATGTTTCAACA

attP11 GTTCAACTTTTTCTATACAAAGTTGGCA (SEQ ID NO:18)  
CAAGTTGAAAAGATATGTTTCAACCGT

attL11 CAACTTTTTCTATACAAAGTTGGCA (SEQ ID NO:19)  
GTTGAAAAGATATGTTTCAACCGT

attR11 GTTCAACTTTTTCTATACAAAGTTGT (SEQ ID NO:20)  
CAAGTTGAAAAGATATGTTTCAACA

---

attB17 CAACTTTTTGTATACAAAGTTGT (SEQ ID NO:21)  
GTTGAAAACATATGTTTCAACA

attP17 GTTCAACTTTTTGTATACAAAGTTGGCA (SEQ ID NO:22)  
CAAGTTGAAAACATATGTTTCAACCGT

attL17 CAACTTTTTGTATACAAAGTTGGCA (SEQ ID NO:23)  
GTTGAAAACATATGTTTCAACCGT

attR17 GTTCAACTTTTTGTATACAAAGTTGT (SEQ ID NO:24)  
CAAGTTGAAAACATATGTTTCAACA

---

attB19 CAACTTTTTCGTACAAAAGTTGT (SEQ ID NO:25)  
GTTGAAAAGCATGTTTCAACA

attP19 GTTCAACTTTTTCGTACAAAAGTTGGCA (SEQ ID NO:26)  
CAAGTTGAAAAGCATGTTTCAACCGT

attL19 CAACTTTTTCGTACAAAAGTTGGCA (SEQ ID NO:27)  
GTTGAAAAGCATGTTTCAACCGT

attR19 GTTCAACTTTTTCGTACAAAAGTTGT (SEQ ID NO:28)  
CAAGTTGAAAAGCATGTTTCAACA

# FIGURE 24C

attB20      CAACTTTTTTGGTACAAAGTTGT (SEQ ID NO:29)  
 GTTGAAAAACCATGTTTCAACA

attP20      GTTCAACTTTTTTGGTACAAAGTTGGCA (SEQ ID NO:30)  
 CAAGTTGAAAAACCATGTTTCAACCGT

attL20      CAACTTTTTTGGTACAAAGTTGGCA (SEQ ID NO:31)  
 GTTGAAAAACCATGTTTCAACCGT

attR20      GTTCAACTTTTTTGGTACAAAGTTGT (SEQ ID NO:32)  
 CAAGTTGAAAAACCATGTTTCAACA

---

attB21      CAACTTTTTTAATACAAAGTTGT (SEQ ID NO:33)  
 GTTGAAAAATTATGTTTCAACA

attP21      GTTCAACTTTTTTAATACAAAGTTGGCA (SEQ ID NO:34)  
 CAAGTTGAAAAATTATGTTTCAACCGT

attL21      CAACTTTTTTAATACAAAGTTGGCA (SEQ ID NO:35)  
 GTTGAAAAATTATGTTTCAACCGT

attR21      GTTCAACTTTTTTAATACAAAGTTGT (SEQ ID NO:36)  
 CAAGTTGAAAAATTATGTTTCAACA

FIGURE 25A

# Vector Assembly Using Modular Vector Element Entry Clones

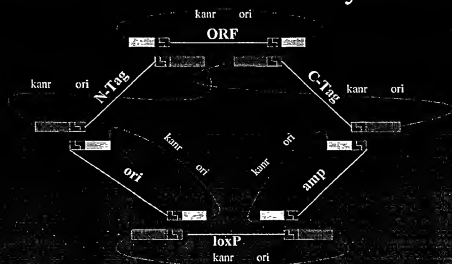


FIGURE 25B

# Vector Assembly Using Modular Vector Element Entry Clones

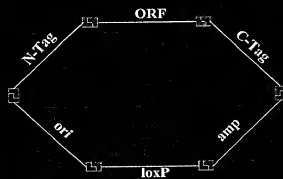


FIGURE 26A

# Construction of attP Plasmids

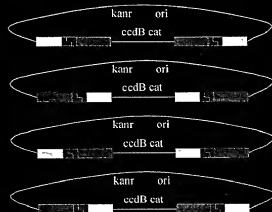
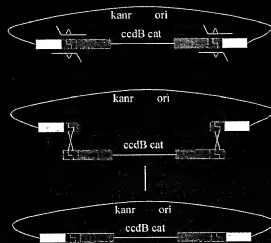
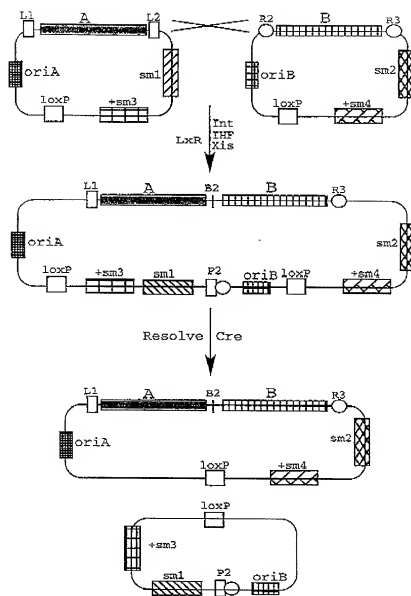


FIGURE 26B

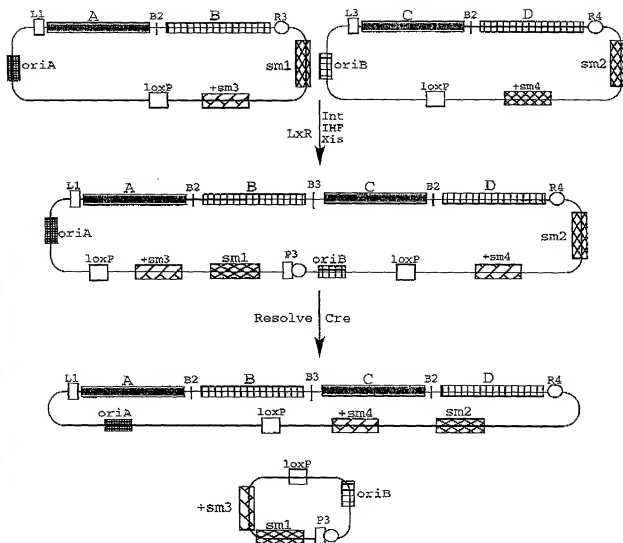
# Construction of attP Plasmids





Transform host that will support replication of *oriA* but not *oriB* and moreover, is sensitive to *+sm3* but resistant to *+sm4*.

FIGURE 27A



Transform host that will support replication of *oriA* but not *oriB* and moreover, is sensitive to +sm3 but resistant to +sm4.

FIGURE 27B